AN INTRODUCTION TO THE TECHNIQUE OF SECTION-CUTTING

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Biol.
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FROM THE NOTES OF
THE LATE MR PETER JAMIESON

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EDINBURGH
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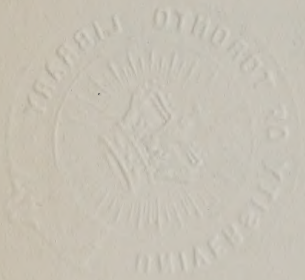

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PREFACE

By Professor J. Graham Kerr, M.A., F.R.S.

In a number of the biological sciences, such as Zoology, Anatomy, Physiology, Pathology, an important part of the technique of the subject is that devoted to the art of section-cutting. Some practical acquaintance with this art is an essential part of the equipment of the serious student of these subjects. We already possess admirable works dealing with such technique—above all, the admirable *Microscopist's Vade Mecum* of the late A. Bolles Lee. I have, however, for many years, felt the need, as doubtless have many other teachers, of a satisfactory, small, elementary book which could be recommended to junior students as giving reliable instruction in the ordinary stock method of cutting sections in paraffin or celloidin while not distracting his attention from these stock methods —a thorough grasp of which is essential as a foundation—by introducing descriptions of the innumerable modifications in details of technique which have been evolved by specialists along particular lines of investigation. Many years ago I urged the late Peter Jamieson—an

accomplished master of microscopical technique—to draw up instructions on such lines, based upon the procedure customary in the department of zoology in the University of Glasgow. Jamieson's personal interests lay, however, more in the direction of the microtome than in that of the pen, and his progress was slow. Now that he has gone, Miss Frances Ballantyne has been so good as to take the matter up, and the fruits of her labour are to be seen in the very lucid and reliable instructions that are to be found in the following pages.

J. GRAHAM KERR.

March 1928.

INTRODUCTION

In those departments of biological science which are concerned with the microscopic structure of the body—Zoology, Anatomy, Histology, Physiology, Pathology—the stock method of investigation is by means of microscopic sections. In the different branches of science alluded to there are differences in detail—particularly in the methods of staining—but the technique in all cases varies chiefly in detail from a common stock method. The object of this little book is to place in the hands of students, at moderate cost, a set of accurate instructions which will enable them to prepare excellent microscopic sections of ordinary tissue. Having learnt the general method, they will then be able to pick up with ease any modification in detail used in specialised work in any one of the departments of science.

Much of the matter given here has already appeared in one or other of the editions of Bolles Lee's *Microscopist's Vade Mecum*, especially the fifth edition, but, for the average student, the methods given by Lee are both too numerous and too scattered. In this book, therefore, only those methods which have been found in practice to

work satisfactorily and which are in general use, are given.

For many years, in the Department of Zoology of Glasgow University, all the microscopic preparations, both whole mounts and serial sections, were made by the late Mr. Peter Jamieson, who was an acknowledged expert in the art of both paraffin and celloidin section-cutting. It was always hoped that he would publish his methods, and, to that end, he was persuaded to make notes of his own particular refinements of the usual methods. Unfortunately, in the spring of 1927, Mr. Jamieson died without having prepared his book, but the methods given here are worked out from his notes and those of Professor Graham Kerr, from which the chapters on the Paraffin Method are almost entirely taken.

AN INTRODUCTION TO THE TECHNIQUE OF SECTION-CUTTING

CHAPTER I

The Standard Method: Introduction

In order to work out the minute structure of the animal body, use has to be made of the microscope and this involves a knowledge of certain methods of technique. It is the object of this book to give an outline of these methods, suitable for students commencing to work with the microscope. A student who has familiarised himself with the elementary methods set forth here will find it a comparatively simple matter to pick up the more specialised methods used in particular departments of microscopic investigation.

With most vegetable tissues, *e.g.* a plant stem, it is possible to cut free-hand sections with a razor, which will show all the ordinary details of structure. It is quite otherwise with animal tissues, the soft protoplasmic cells of which are without the firm supporting cell-wall that is

present in plants. Consequently, it is necessary, before attempting to cut sections of the soft animal tissues, to infiltrate them with some solid, supporting material, of such a consistency that it can be cut into fine sections. The substances now—after much experience—practically invariably used for this purpose are: (1) solid paraffin of high melting point; and (2) celloidin—a substance allied to gun-cotton and celluloid.

Paraffin and Celloidin Methods

Of these two methods the most commonly used is the paraffin method, for it is both quicker and easier than the celloidin method. By it, also, thinner sections can be obtained than by the celloidin method, and for most small objects there is no doubt that it is the most satisfactory. It is also the best method to start with, in learning the technique of section-cutting. It has, however, various disadvantages. Large and tough specimens will not cut properly when embedded in paraffin, because the paraffin is not sufficiently firm to hold such objects together. The tissues shrink somewhat during the process, so that the different structures become slightly separated from one another. In some cases this brings out points which would otherwise escape observation, but it is well to remember that the separation of the different tissues is artificial. Another disadvantage of the method is that heat has to be

employed in the embedding process, which, of course, tends to cause alteration in the consistency of the tissues.

The celloidin method, on the other hand, is a much more lengthy process, but it has the advantages of not causing shrinkage and of not requiring heat. By it, also, large and tough objects, which will not cut by the paraffin method, can be sectioned quite satisfactorily. It is not possible, however, with celloidin embedding to get very thin sections. Where possible, with detailed work, it is well to have parallel series of sections prepared by the paraffin and the celloidin methods.

Preliminary Processes

For both methods the specimen to be sectioned has to be carried through certain preliminary processes to prepare it for the infiltration of the embedding substance. As these processes are the same, whichever method is used for the section-cutting, the remainder of this chapter will be devoted to a general description of them.

Fixation

The first of the chain of processes preliminary to the actual cutting of sections, is what is known as fixation. This consists of the killing of the protoplasm by some very rapidly acting poison, which, at the same time, coagulates and hardens the

protoplasm in as nearly as possible the position which it had in life. The special object of the process of fixation is not, as is often stated, to preserve the protoplasm in a condition as near as possible to that of life—for, of course, the mere fact of not being alive separates preserved protoplasm from living protoplasm by an immense gulf—it is rather to provide a standard process which allows of the useful employment of the comparative method. As soon as protoplasm dies there commences a series of post-mortem changes so complex, so little undertsood, so variable in the rate at which they proceed, as to make it a task of extraordinary difficulty to interpret the minute details of cell structure seen in different preparations. On the other hand, by subjecting the living tissues to a standard process of fixation, it becomes possible to employ the comparative method, even though we are still unable fully to interpret the appearances we observe.

Perhaps the best fixative for the beginner to use is a saturated solution of corrosive sublimate in water. It, however, possesses the fault that it causes a considerable amount of shrinkage to living tissues placed in it. This may be counteracted by the addition of a certain quantity of Acetic Acid, a substance which possesses the opposite fault, that of causing swelling of tissues subjected to its influence. A good standard fixing solution is made by mixing 5 cc. Glacial Acetic Acid with

95 cc. of a saturated solution of Corrosive Sublimate in water. The specimen, with its tissues still in the living condition, should be placed in the solution and left until thoroughly fixed throughout—the time necessary for this varying with the size and permeability of the specimen. To ensure thorough fixation, it is well to allow a considerable margin of time; thus, small specimens, up to, say 10 mm. in diameter, may be left a couple of hours in the fixing fluid. A necessary precaution to be borne in mind when using corrosive sublimate is to avoid letting steel instruments come in contact with the fluid, as this causes precipitation. When it is necessary to manipulate the specimen, so long as there is any corrosive sublimate about it, splinters of glass should be used instead of metal needles or section-lifters.

Removal of Fixing Agent

The process of fixation having been completed, the next thing to do is to remove all trace of the fixing agent. In the case of corrosive sublimate, this is satisfactorily accomplished by the soaking in baths of alcohol of gradually increasing strength, which constitute the next process, that of dehydration, so that the two processes are combined. It is necessary to prolong the baths of alcohol until all trace of the corrosive sublimate has been removed, renewing the alcohol from time to time until it

ceases to show the presence of corrosive sublimate. A convenient indicator to use for this purpose is tincture of Iodine. This should be added to the alcohol bath when the strength of 70 per cent. is reached, until the alcohol is of a port-wine colour. This colour gradually disappears so long as any corrosive sublimate is present. When the persistence of the iodine colour shows that the corrosive sublimate has been got rid of, the iodinised alcohol should be poured off and replaced by ordinary 70 per cent. spirit, which will remove the brown stain produced by the treatment with iodine. In case any deposit of bright yellow mercuric iodide has been formed on the surface of the specimen, it must be put back into iodinised alcohol, the mercuric iodide soon disappearing in the presence of excess of iodine. It may be explained that the reason for taking pains to get rid of the corrosive sublimate in the specimen, is that otherwise it forms crystals which may interfere with the process of section-cutting or, on the other hand, may make their appearance later on in the form of crystallites resembling minute pins when seen under the microscope.

Dehydration

The standard method of section-cutting involves the specimen being permeated with paraffin (or celloidin), a substance which will not mix with water. It is therefore a necessary prelimin-

ary to remove all trace of water. This process of *dehydration* is carried out by soaking in successive baths of 30, 50, 70, 90, 100 per cent. Alcohol. The percentages may be made up by using absolute (*i.e.* anhydrous) alcohol; *e.g.* 30 per cent. is prepared by adding 30 cc. absolute alcohol to 70 cc. water. Owing to the very high price of absolute alcohol it is more economical to use what is known technically as "pure spirit." This contains a varying percentage of water and can be diluted further by adding water until the desired percentage is shown by a Tralles Alcoholimeter. This latter, which can be purchased for a few shillings, is a specially graduated hydrometer, and as it floats vertically it is necessary to make the mixture in a tall jar. The 100 per cent. alcohol is bought as "absolute alcohol." Great care must be taken not to expose it to the air as it at once absorbs moisture. The specimen, while in it, should be enclosed in a tightly corked tube. It is well to pour off the absolute alcohol after a time and replace it by a fresh supply to make sure of getting rid of the last trace of moisture.

The duration of the alcohol baths will depend upon the size and permeability of the object. For a small object, such as a 42-hour chick embryo, twenty minutes may be allowed for each of the weaker alcohols, but it is best to leave the specimen overnight in a second bath of 90 per cent. and then give it two successive baths

of absolute alcohol of about twenty minutes' duration.

For keeping objects for a prolonged period, alcohol of about 70 per cent. may be used. Some workers prefer stronger alcohol, but if the strength is much over 70 or 75 per cent. the object is apt to be rendered brittle. On the other hand, the alcohol should be tested from time to time, and, if necessary, replaced to make sure that its strength does not get much below 70 per cent., as, in this event, the specimen is apt to become macerated. But it should be noted that, however great care be taken, specimens in alcohol always undergo a slow process of degeneration. For very fine investigations, therefore, one always tries to obtain fresh material.

CHAPTER II

Paraffin Method : Embedding

The preliminary processes of fixation, washing and dehydration, which are common to all methods of section-cutting, having been completed, the specimen is now ready for the specialised treatment of each particular method. In this and the following chapters, details are given of the Paraffin Method.

Clearing

Having now completed the process of dehydration, it is necessary to soak the specimen in some fluid which will mix more readily with paraffin than is the case with alcohol. Many fluids are suitable for the purpose, such as Chloroform, Xylol, or Oil of Cedar-wood. The last-mentioned is one of the best, but Xylol is also very good, and, as it is easier to use, is consequently to be recommended to the beginner.

The specimen is transferred from the absolute alcohol to the clearing agent and left therein until completely permeated. As it becomes permeated it assumes a transparent appearance, and,

hence the name " clearing " given to this process. Certain precautions are necessary to obtain the best results.

1. The transference to the clearing agent should be effected as gradually as possible. The best method of ensuring this is to draw off most of the absolute alcohol, leaving just sufficient in the bottom of the specimen-tube to cover the object completely, and then to allow the clearing fluid to run slowly in beneath the absolute alcohol from a dipping tube held vertically, with its lower end close to the bottom of the specimen-tube. The less dense absolute alcohol containing the object will be carried up on the surface of the denser clearing fluid. The object will float at the plane of separation of the two liquids, but will gradually sink down as the clearing fluid passes into it. As soon as it has sunk completely into the clearing fluid, the absolute alcohol should be carefully pipetted off and the object left to soak in the clearing agent.

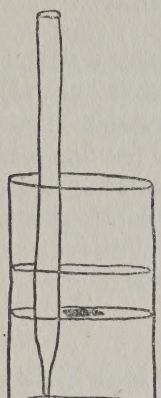

FIG. 1—Diagram to show method of transferring the object from absolute alcohol to the clearing agent.

2. Care should be taken to give the specimen a prolonged bath in the clearing fluid. It had better be left for a considerable time after it has assumed the clear transparent appearance. It

is, in fact, a good plan to prolong the clearing over-night, except in the case of very minute objects. It is well to replace the clearing fluid once or twice, to make sure that there is no considerable admixture of absolute alcohol.

Paraffin Baths

The specimen is now ready to embed in paraffin, the first process being the infiltration by the melted paraffin. This process is accomplished in the following way. The object saturated with the clearing fluid is placed in a bath of paraffin, kept just above its melting point, for such time as is necessary to allow the paraffin to replace the clearing agent completely. It is then allowed to cool, so that it is enclosed in a solid block of paraffin, which, with its contained specimen, may be cut into sections.

The following are the details of the process: (1) The paraffin should be of the best quality, translucent and bluish in colour, giving out a slightly metallic ring when dropped on a wooden table, and with a melting-point of about 52° C. (2) The bath of paraffin in which the object is soaked must not be allowed to rise appreciably above the melting point, as heat affects the tissues harmfully. The simplest arrangement for securing this is to have the paraffin in a metal trough (say a tin box, $4 \times 2\frac{1}{2} \times 2$ inches), resting on a small metal table, heated at one end by a small

flame. By sliding the trough about on the table a position can easily be found such that the paraffin remains completely melted at one end of the trough, while it remains completely solid at the other end. Between these two portions is an inclined plane of solid paraffin, and, if the specimen is laid upon this, it may be depended upon not to rise in temperature appreciably above

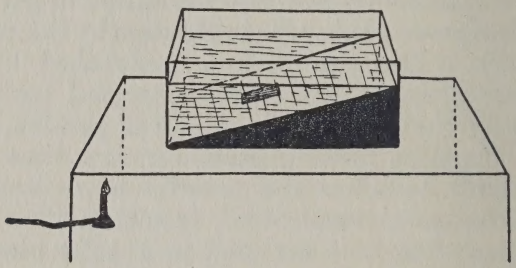

Fig. 2—A simple paraffin bath.

the melting point of the paraffin, so long as it rests upon the surface of the unmelted paraffin. An advantage of the apparatus just described is its simplicity and its portability. In a regular laboratory, water-jacketed ovens or water-baths kept at the proper temperature by a thermostat will be available, and small vessels of copper, preferably coated with nickel internally, can be used to contain the objects while soaking in paraffin. As regards thermostatic arrangements, the most efficient and reliable, in our opinion, is the simple and inexpensive gas-regulator of Reichert, which

can be purchased at any chemical dealers. (3) The duration of the paraffin bath should be the minimum necessary to secure proper penetration. In the case of such a small and permeable object as a second-day Fowl embryo about twenty minutes is sufficient. (4) Care must be taken that the paraffin does not contain any appreciable admixture of the clearing fluid. As soon as it becomes apparent that this is the case the paraffin should be replaced by a new supply.

Preparation of Block

When completely penetrated, the specimen is transferred by means of a section-lifter, warmed in a Bunsen flame, to a small tank of paraffin, which is allowed to solidify. The most convenient tank is made by two L-shaped pieces of heavy metal or glass, which can be laid on a flat plate of metal or glass in such a position as to enclose a space large enough to accommodate the specimen with a considerable volume of paraffin all round it. The space is filled with melted paraffin by means of a *wide-mouthed* pipette, warmed in the Bunsen flame, care having been taken to smear the inner surface of the L's and also of the basal plate with a very thin layer of glycerin or vaselin to prevent the paraffin from adhering to them. As soon as the bottom layer of paraffin is seen to solidify, the specimen is taken up with the section-lifter, warmed as before,

and laid in the melted paraffin. If the object is elongated in form, as, for example, a piece of a worm, it should be arranged with its long axis parallel to the long axis of the tank of paraffin. For adjusting the position of the object in the melted paraffin a pair of needles mounted in

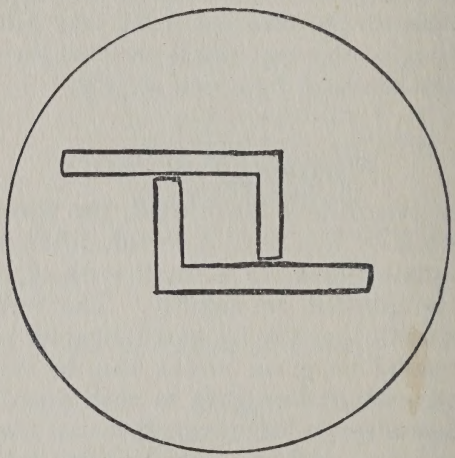

FIG. 3—A tank for the preparation of the paraffin block.

wooden handles should be used, the needles being first heated in the Bunsen flame to prevent their becoming coated with solidified paraffin.

A large dish of cold water should be at hand, and, as soon as the specimen is properly in position in the paraffin, the basal plate with its L's should be carefully lifted up and lowered on to the surface of the cold water, so as to cause the paraffin

to solidify rapidly from below upwards. The whole should be kept with the plate just touching the water until the paraffin is solidified throughout and then submerged completely so as to complete the cooling of the paraffin as rapidly as possible. If the paraffin is not cooled rapidly it is apt to crystallise and not give the proper consistency for cutting. On the other hand, if submersion in the water takes place before the paraffin is solidified throughout, the still liquid paraffin suddenly solidifies, and its shrinkage forms a cavity which, by the rupture of the hard outer layer, becomes filled with water. A properly prepared block, solidified from below upwards, should be perfectly homogeneous, its upper surface marked by a deep depression where the still soft upper layer has been drawn downwards by the shrinkage of the paraffin underneath.

It is important, in making the block, to have the tank considerably larger than the object, for two reasons:

1. To allow sufficient spare paraffin for the block to be trimmed down to the size and shape best suited to the kind of sections required.

2. To prevent the deep depression formed on the upper surface from being so deep as to expose the object. The smaller the tank, the deeper, comparatively, the depression.

CHAPTER III

Paraffin Method: Cutting and Mounting Section-Cutting

In dealing with plant tissues it is customary to cut a number of free-hand sections, and from them to choose the thinnest and most complete for examination. In the case of animal tissues, however, the cell structure is commonly on a much smaller scale than it is in plants, and therefore it is necessary to make much thinner sections, for sections should normally be of an order of thickness corresponding with the size of the cell-units. Further, in studying the structure of minute animals, with their complicated arrangement of organs, it is necessary to use the *serial section* method, in which the animal is converted into a series of sections, uniform in thickness, which are mounted in correct sequence, so that the whole anatomy of the creature may be worked through from end to end. For both these reasons the free-hand method of cutting sections has to be replaced by a mechanical method which will secure sections of the necessary degree of thinness and uniformity.

Microtomes and Knives

The instruments used for this purpose are known as *microtomes*. Of these a great variety exist, from a simple instrument costing a few shillings up to the beautiful Giltay microtome costing many pounds. As an example of a good microtome of moderate cost we will take the

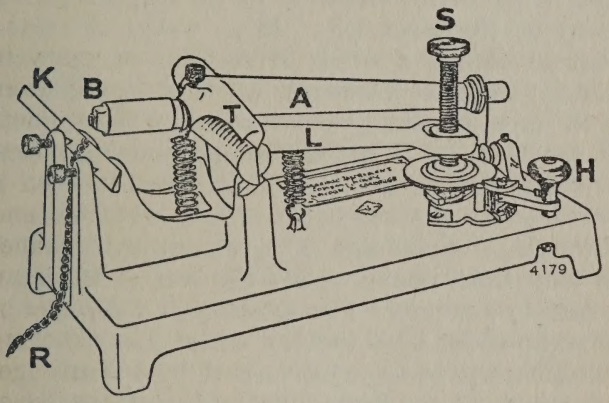

Fig. 4—The Cambridge "Rocker" microtome.

Cambridge "Rocker" microtome, illustrated in Fig. 4. In this instrument the knife (K) is fixed in position by the two screws. The object in its block of paraffin is fixed to the brass holder (B), which fits tightly on to the shorter end of the swinging arm (A), supported on trunnions (T). The oscillations of this arm, brought about by the pull of a cord, working against that of a strong

spiral spring, cause the block of paraffin to be carried up and down past the edge of the knife. In order to cut uniform sections of a given thickness, it is obvious that the paraffin block must be pushed forwards through the necessary distance before each downward movement. This is effected by the block of metal which carries the trunnions in V-shaped incisions at its upper end being tilted forwards by an upward movement of the lever (L). It is clear, therefore, that to obtain a series of sections of approximately uniform thickness, all that is necessary is to have an arrangement whereby the far end of the lever (L) is raised through equal amounts between the strokes. Such an arrangement is provided by the screw (S), which, when rotated through a given arc in a clockwise direction as seen from above, raises the end of the lever a certain amount. The rotation of the screw is brought about by a toothed wheel, into the teeth of which a pawl engages, carried by a small arm (H), which, at the same time, pulls down the main swinging arm, carrying the paraffin block.

Variation in the thickness of the sections is brought about in the following manner. Beneath the toothed wheel and concentric with it is a metal arc which can be moved stiffly upon its axis. This projects beyond the periphery of the toothed wheel sufficiently far to keep the pawl from engaging in the teeth. If the arm carrying the pawl is pulled forwards, as soon as the pawl

passes over the edge of the metal arc, it is pulled against the edge of the toothed wheel by the action of a small spring. The pawl now engages, and, for the remainder of the arc through which the arm is pulled, the toothed wheel is pulled round with it. The toothed wheel is therefore rotated through a definite amount, and, as a consequence, the paraffin block is fed in towards the knife a definite amount. This amount, and therefore the thickness of the sections, will obviously depend on the distance through which the stiffly moving metal arc has been pushed back from the check-pin, which limits the forward movement of the handle. In the earlier makes of the microtome, one estimated the thickness of the section by the number of clicks heard as the pawl slipped back over the teeth of the wheel before it reached the metal arc—sixteen teeth being the equivalent of 10μ [1] thickness of section. In the more modern makes a graduated scale on the metal arc enables the thickness to be read off directly.

The most convenient knife to use is an ordinary razor which, however, must not be hollow-ground.[2] It must have the finest possible edge. It should be carefully " set " on a fine-grained and perfectly flat oil-stone, the blade being held flat down on the stone with light pressure and moved with the edge foremost. (It is advisable to have

[1] 1μ =the thousandth part ($\frac{1}{1000}$) of a millimetre.

[2] A specially made section-cutting razor can, however, be obtained.

two oil-stones or hones, one of slightly coarser grain for the preliminary sharpening, the other for finishing off. A little paraffin should be mixed with the oil to give the stone a better grip. The stone should be cleaned frequently with strong ammonia, and it is a good plan to keep the two stones in contact and occasionally to give them a rub against each other to keep the surface flat.) Before using the razor, the edge should be examined with the low power of the microscope, to see that it is perfectly uniform and free from saw-like irregularities. The knife should then be cleaned carefully with absolute alcohol.

Attachment of Block to Microtome

The mass of paraffin containing the object is trimmed down carefully, until it forms a block with accurately rectangular sides. The block-carrier is removed from the microtome and the block fixed to it by heating a flat spatula in the Bunsen flame, and, pressing the block on to the paraffin of the block-carrier, withdrawing the hot spatula from between the two paraffin surfaces, so that they fuse into a continuous mass. If the object is to be cut in a definite direction, such as, for example, a piece of worm to be cut into transverse sections, care should be taken to adjust the block on the holder exactly before its attachment cools and becomes rigid. Care should be taken also, that the block is not too long for the available distance of back-and-forward travel of the

carrier, imposed by the limited height of the elevating screw (s).

Cutting

Before commencing section-cutting, the microtome should be adjusted to cut sections 10μ in thickness, and the elevating screw should be turned backwards to its limit. The brass carrier with its block is then moved forwards until the paraffin block *just* clears the edge of the knife.

Sections are now cut by moving the handle backwards and forwards. This should be done gently: it should not be drawn violently forwards so as to bang against the check-pin. It should be allowed to spring back rapidly or slowly according as a quick movement or a slow movement of the block past the razor is found to give the best sections of the particular object.

The beginner will probably find that his first sections are by no means perfect. The sections may be scored by vertical lines or grooves; the grooves may be so deep that the section splits into two or more pieces. Any such effects are due to irregularities in the cutting edge of the razor. The razor should be moved lengthwise so as to bring a different portion of the blade into action. If it proves impossible to find a portion of the edge free from flaws, the razor must be reset or replaced by another. The splitting of the sections may be caused by some of the

paraffin sticking to the razor edge, or by a bristle or some fine fibre attached to the block.

Again, the section, as it is cut, may, instead of remaining flat, curl up into a tight roll. The meaning of this is that the sections are being cut at too great a thickness for the prevailing room-temperature, and the difficulty may be overcome by cutting thinner sections. If, for any reason, it is desired not to deviate from the 10μ thickness, then the same result may be obtained by slightly raising the temperature, *e.g.* by the proximity of a lamp. If the razor is too blunt, this bluntness may be the cause of the curling of the sections.

Lastly, the sections may look quite flat, but their vertical breadth, as they hang on the razor, may seem to have undergone diminution, so that if sections are being made of a square block, the sections are no longer square. If the cause of this is looked into, it is found that the section is not really flat, but traversed by innumerable minute wrinkles, parallel to the edge of the knife. This means that the sections are too thin for the prevailing room temperature. It may be corrected by increasing the thickness of the section or by cutting the sections at a lower temperature. A fine edge on the knife will also diminish the tendency to wrinkle.

These little difficulties having been surmounted, sections may now be cut rapidly one after the other. Frequently, especially if the temperature be fairly high, it will be found that consecutive

sections adhere together, so that they come off the microtome as a continuous ribbon. A great deal of time and trouble is saved during the subsequent operations when this is the case, and consequently, when a continuous series of sections is desired, it is usual to take means to ensure that the sections will "ribbon." This is done by coating the block, after it has been trimmed and fixed to the holder, with a thin layer of soft sticky paraffin of a much lower melting point (45° C.) than that used for the actual embedding. The block on its holder may be dipped for a moment into a bath of melted paraffin of the lower melting point, or the upper and lower surfaces of the block, when it is in position on the carrier, may simply be painted over with the super-heated soft paraffin by means of a camel-hair brush.

During the process of cutting, the end of the ribbon is held in forceps or supported by a glass rod with the left hand, while the microtome is manipulated with the right. When the length becomes unwieldy, the ribbon is cut through near the microtome and the detached portion laid on smooth white paper, care being taken to have the dull side of the sections uppermost, the smooth glossy surface next the paper. It will be found that, whenever possible, it is best not to break the ribbon at the razor edge, but rather where it touches the paper. The attached piece of ribbon is held up again with the forceps or brush, while more is cut, until it is again long

enough to stretch the whole length of the paper. In this way straight rows of ribbon are laid along the paper, and there is less danger of losing any of the sections. If, on the other hand, the ribbon is completely removed from the razor, it is next to impossible to keep every section, for where the new ribbon is started, the first sections are very apt to become separated or to roll up, whereas, if part of the ribbon is left on the razor, the fresh sections simply attach themselves to it and form an extension to the ribbon.

Mounting

The object having been converted into a series, we will suppose a ribbon, of properly prepared sections, the next step consists of the mounting of these in Canada balsam for permanent preservation. Sections of small size are conveniently mounted on glass slides of the standard size (3×1 inches). When they form a continuous series they are conveniently arranged under an oblong coverslip (say $2 \times \frac{7}{8}$ inches) in the manner illustrated by Fig. 5. The sections are arranged in transverse rows and are placed towards one end of the slide, so as to leave a considerable space at the other end for the label, and also to facilitate the manipulation of the slide under the microscope by the fingers of the left hand. When it is important that the sections, as seen under the microscope, should be orientated in a

particular direction, this should be borne in mind before cutting the sections. Thus, if a small worm is being converted into transverse sections, the paraffin block should be so trimmed and arranged that the dorsal side is below when the block is fixed to the microtome ; if this is done, the dorsal side of each section will be towards that edge of the slide which is below in Fig. 5,

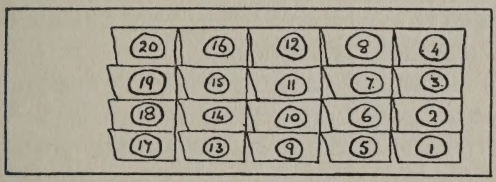

FIG. 5—Diagram to show the method of arranging the sections on the slide.

and, when the slide is placed on the stage of the microscope, label to the left, the sections will be seen the right way up, owing to the compound microscope inverting the image.

The slide is first carefully cleaned and coated on one side with a layer of adhesive egg-albumen, which will serve to cement the sections in position. A little of the albumen should be placed on the slide with a glass rod, smeared over the surface, and then rubbed off almost completely with the finger, as it is necessary that the layer of albumen should be exceedingly thin.

The slide is now laid on a piece of white paper or card, upon which has been drawn the outline

of the slide, with that of the coverslip in the required position to serve as a guide in arranging the sections. A piece of ribbon, consisting of as many sections as will fit comfortably into the width of the slide (four, in the case illustrated in Fig. 5) is cut off with a sharp scalpel, lifted with forceps or by a spear-headed needle with a rather rounded point, and laid in the proper position on the slide. A second piece of ribbon is laid alongside the first, and so on, until the whole area of the coverslip is filled up.

The sections are now flattened by floating them on water heated to a temperature high enough to soften the paraffin and allow the wrinkles to straighten out. By means of a pipette, sufficient cold water is run underneath the sections to cause them to float on a continuous pool on the slide. The slide is now gradually warmed by laying it on the warmed metal table or the top of the water-bath. It is watched carefully, and the sections are presently seen to expand somewhat as the wrinkles flatten out, the paraffin at the same time assuming a more transparent appearance. The slide is now lifted off the warmed plate, any sections that may have become displaced are slid back into their place, and the water is allowed to drain away by tilting the slide very carefully, holding the sections in place until they are stranded on the albuminised surface. The slide is now laid aside in a sloping position, the sections underneath, so that they do not receive falling

dust. After the slide is absolutely dry—the time necessary for this will, of course, depend upon the dryness of the atmosphere—in case of doubt the slide may be immersed in absolute alcohol to make certain—it is placed in a jar of xylol until the paraffin has completely disappeared. The final step is to drain off the xylol (but on no account must the sections be allowed to *dry*), place a drop of Canada balsam over the sections, and cover with a coverslip. (*N.B.*—If the sections are to be stained on the slide, this will, of course, be done before the Canada balsam is applied. See next chapter.)

Where a long series of sections is being mounted, care should be taken to number the individual slides, either permanently by means of a writing diamond or temporarily by means of a greased pencil or bit of hard kitchen soap.

The Canada balsam should be a fairly fluid solution in xylol. The Canada balsam should be drawn lengthwise along the centre of the coverslip with a glass rod, and the coverslip then should be lowered gently from one end, so that the balsam flows out evenly over the sections towards each side of the coverslip without forming air-bubbles. The slides should be left horizontal for a few days to enable the balsam to harden at the edges, otherwise the coverslip is apt to slide off.

CHAPTER IV

Paraffin Method: Staining

A preparation made according to the preceding directions would, in most cases, be practically useless, for the sections would be practically invisible owing to the intense clearing power of the highly refracting Canada balsam. To obviate this it is necessary to have the sections stained with some kind of dye. The reason for omitting the process of staining from the scheme of operations described so far, is that it may be introduced at either of two different stages. The specimen may be stained " in bulk " before being embedded in paraffin, or the sections may be stained after being fixed upon the slide and deprived of the paraffin. A great variety of different stains are in use, some better suited for staining in bulk, others for staining on the slide. The finest results are obtained by staining on the slide, but as staining in bulk is simpler it is best for the student to use it to begin with. He is advised to use Borax Carmine stain, prepared according to the following directions:

Borax Carmine

Dissolve 4 gms. of Borax in 100 cc. of warm water. Add to the solution 2 gms. of Carmine and boil for half an hour, then let it stand for 24 hours and add 100 cc. of 90 per cent. alcohol. Filter after a couple of days.

Staining in Bulk

The staining is intercalated in the process of dehydration. After the object has been thoroughly freed from any remains of the fixing agent, it is placed in stain until completely permeated. The exact time necessary will depend upon the size and degree of impermeability of the object. In the case of a small, fairly permeable object, it is well to leave it in stain overnight, and then to wash out the excess of stain.

It should be taken as a general rule that the best results are obtained by over-staining and then washing out the excess of stain, for it is found that different types of cell, and different parts of the individual cell, differ greatly in the tenacity with which they retain the stain, so that the various details of structure stand out distinctly on account of their varying depths of colour.

If the stain used is Borax Carmine, the fluid used for the washing-out process is Acidulated Alcohol. (To each 100 cc. of 70 per cent. alcohol

add six drops of strong Hydrochloric Acid.) The stain in which the object has been immersed is poured off and replaced by acidulated alcohol. As soon as this takes on a distinctly red colour it is replaced by a fresh supply, for the acidulated alcohol will only fulfil its function properly if it is practically colourless. The treatment with acidulated alcohol should not be stopped so long as any colour comes away. A 42-hour chick embryo, left over-night in stain, should be kept for about two hours in several changes of acidulated alcohol.

After completion of the process of staining and washing out, the series of processes is resumed at the appropriate point. In the particular case described, the object at the end of the washing-out process is in alcohol of 70 per cent. strength. Logically the next step would be to transfer it to 90 per cent. alcohol, but, before doing this, it is best to treat it with plain 70 per cent. alcohol to get rid of the Hydrochloric Acid of the acidulated alcohol. Treatment with 90 per cent. absolute alcohol, xylol then proceeds as described.

If, as sometimes occurs, it is found that the sections when cut are not sufficiently deeply stained, it is possible to restain by taking the slide down through absolute, 90, 70 per cent. alcohol, and then to a tube of stain for a few minutes. The slide is then washed with acid alcohol, ordinary 70, 90 per cent. and absolute alcohol,

followed by a bath of xylol, and then the slide is mounted with Canada balsam as described at the end of the last chapter.

When the specimen is stained in bulk and the slides are to be mounted directly, it will be found best to put them for a few seconds into absolute alcohol, after the paraffin has been dissolved out with xylol. Then they are put into a fresh tube of xylol for a few moments and mounted in Canada balsam. The reason for this bath of absolute alcohol is to wash out all trace of the glycerin from the albumen used to cement the sections to the slide. If there is any glycerin left, it is apt, after a time, to form little round drops all over the surface of the sections, and thus spoil the preparation.

Staining on the Slide

When the sections are to be stained on the slide, and the best results are obtained by this method, the slide, after the paraffin has been removed with xylol, is given a few minutes in absolute alcohol, then in 90, 70, 50, and 30 per cent. alcohol. A convenient general stain for use at this stage with paraffin sections, is Ehrlich's Hæmatoxylin. The slide is transferred from 30 per cent. alcohol to the hæmatoxylin for about five or ten minutes, washed with 30 per cent. alcohol to take away the excess stain, and then dehydrated in the usual way. If a counter-stain

is wanted to bring out the cytoplasm in strong contrast to the nuclei, a dip for about half a minute in Eosin (a saturated solution of eosin in absolute alcohol, diluted with twice its volume of plain absolute alcohol), after the slide has been completely dehydrated, gives satisfactory results. The eosin is washed out with absolute alcohol, and the slide is cleared in xylol and mounted in Canada balsam.

Many different stains are used for different purposes, but for an ordinary (cytoplasm) stain, either of the two methods given here works satisfactorily: Borax Carmine for staining in bulk, and, for staining on the slide, Ehrlich's Hæmatoxylin, with a counter-stain with Eosin. Both stains are obtainable from dealers ready made up.

Ehrlich's Hæmatoxylin

Dissolve 2 gms. Hæmatoxylin in 100 cc. Absolute Alcohol, and 2 gms. Ammonia Alum in 100 cc. Distilled Water. Mix the two solutions and add 100 cc. Glycerin and 10 cc. Glacial Acetic Acid. Leave exposed to light for at least a month, then filter and keep in a stoppered bottle.

CHAPTER V

Paraffin Method : Miscellaneous Hints

The student should first of all master the ordinary method of section-cutting, described in the preceding chapters. When he has made himself efficient in cutting sections by that method, he will find the following additional remarks useful.

Fixing Agents

The Corrosive Sublimate and 5 per cent. Acetic solution is an excellent fixative for general purposes. It can be used with fresh or salt water and either hot or cold. It gives good results for histological preparations. Very delicate specimens need only a few seconds, while large objects should be given from some minutes up to some hours, according to their size and permeability. For small fresh-water forms, it is best to dilute the solution of corrosive sublimate with an equal volume of water. For marine animals the concentrated solution is required, and for such things, as, for example, coral polyps, it is best to use a heated solution. Always wash out with 50 per cent and then 70 per cent. alcohol, and test

with a solution of Iodine in 70 per cent. alcohol, to make sure that all the corrosive sublimate is removed.

Various other fixing agents are in use for different purposes, as for example :—

Flemming's Solution (Strong)

1 per cent. Chromic Acid . .	15 cc.
2 per cent. Osmic Acid . .	4 cc.
Glacial Acetic Acid . . .	1 cc.

This solution is good for fixing nuclei and chromosomes, but it does not keep well and penetrates badly, so that only small pieces of tissue or small animals can be fixed with it. The specimen should be given from 1 to 24 hours in the fixing agent (12 hours is the usual time), washed in running water for some hours, and then put into 30 per cent. alcohol.

Bouin's Solution

Saturated solution of Picric Acid in water	75 cc.
40 per cent. Formalin . . .	25 cc.
and, at the time of using,	
Glacial Acetic Acid . . .	5 cc.

This is a good fixing agent and penetrates well. The specimen must not be left in it longer than 18 hours (the usual time is from 6 to 12 hours).

Wash out with 50 per cent. alcohol, *not* with water.

Zenker

Mercuric Chloride (Corrosive Sublimate)	5	gms.
Potassium Bichromate	2·5	gms.
Sodium Sulphate	1	gm.
Distilled Water	100	cc.
and, at the time of using,		
Glacial Acetic Acid	5	cc.

This fixing agent does not keep well. The specimen should be left in it for from 3 to 12 hours, washed in running water for some hours, and then transferred to 30 per cent. alcohol. Having been taken through 30, 50, and 70 per cent. alcohol, it should be treated with a solution of Iodine in 70 per cent. alcohol to make sure that all the corrosive sublimate has been removed.

Osmic Acid

1 per cent. Osmic Acid in Water

This fixing agent is not so much used as in the past. It kills suddenly and hardens animals, preserving their transparency if they are not left too long in it. The specimen should be washed in distilled water whenever it begins to turn a little brownish. Osmic acid attacks objects quickly, and should only be used with small specimens,

as it hardens the surface-layers rapidly, and does not penetrate very far. If the specimen is immersed in it too long it becomes brittle. Osmic acid blackens the fat in tissues, but this can be removed from the preparation by giving it a bath of xylol. After fixing, the specimen must be washed for about 15 or 20 minutes in running water and then treated with 50 per cent. alcohol and two washes of 70 per cent. alcohol.

Acetic Acid

Acetic acid kills rapidly, and with contractile animals it leaves them fairly well extended and transparent. A weak solution is sometimes used, but the best results are obtained with concentrated solutions. It acts rapidly, and contractile and delicate animals are apt to be softened if they are left too long in it; 3 to 5 minutes in a large quantity of the acid is long enough—very delicate objects require only 1 minute, and, in the case of Planarians, it has been found that momentary action is necessary, *i.e.* a flood of acetic acid, followed instantly by a flood of 50 per cent. alcohol to wash it away. Wash out with 50 per cent. alcohol, then 70 per cent. Repeated washing is needed to make sure that all the acid has been removed.

Formalin

4 per cent. Formalin (1 vol. commercial Formol in 9 vols. Water)

This is sometimes used as a fixing agent, but it is not very satisfactory except for such objects as the eggs of frogs and newts. The object should be left for at least 2 days in formalin and then washed out with alcohol. Formalin is one of the best preserving fluids for keeping specimens for a prolonged period.

Dehydrating

If a piece of freshly killed tissue (unless of very small size) were placed directly in absolute alcohol there would be great shrinkage, and much destruction would be done to the delicate details by the violent diffusion currents set up. Hence the custom of bringing about the transference to absolute alcohol by a series of small steps, using grades of alcohol of gradually increasing strength. It is best to carry out the process of dehydration in a short and wide-corked tube (say $1\frac{1}{2} \times 1$ inches), and, where the object is delicate or contains cavities, care should be taken to keep it submerged, leaving a sufficiency of the preceding grade of alcohol to cover it each time the alcohol is changed. In the case of very delicate objects, it is well to intercalate a bath of 80 per cent. alcohol

between the 70 per cent. and the 90 per cent. To ensure the dehydration being complete, the bath of absolute should be renewed once or even twice. It must be remembered that absolute alcohol rapidly absorbs moisture from the air, and great care should be taken not to expose it for longer than is absolutely necessary.

Clearing

Xylol has been recommended as being one of the best all-round clearing agents. Cedar Oil is, in some respects, better, but it has the disadvantage that minute traces of it render the consistency of the paraffin unfavourable for section-cutting, and this makes it necessary to transfer the object to a second or even a third bath of paraffin in order to get rid of the cedar oil.

The essential points to bear in mind in regard to the process of clearing, are :

1. It is at the transference from alcohol to the clearing agent that shrinkage and distortion are more likely to occur than at any other stage in the process, and consequently it is of utmost importance to make this transference as gradually as possible.

2. It is essential to see that the alcohol is quite completely replaced by the clearing agent. To ensure this, it is advisable to replace the clearing agent by a fresh supply twice, and it is a good

plan to leave the object over-night in the third bath.

A combination of cedar oil and xylol gives quite satisfactory results. The object is first cleared with cedar oil, which does not so easily cause shrinkage as does xylol, and when the specimen is completely cleared, the cedar oil is replaced by xylol, which mixes better than cedar oil with paraffin.

Embedding

The quality and melting point of the paraffin is of great importance. For general purposes, in a temperate climate, paraffin with a melting point of 52° C. is the most suitable. It should be transparent, of a bluish colour, and should give a metallic ring when a slab of it is dropped upon a table. For cutting thick sections, 30–40μ or more, a softer paraffin should be used, with a melting point of 40°–45° C.

While the simple hot-plate arrangement, mentioned in Chapter II, is eminently suitable for class-work or for work away from laboratory resources, it is more convenient, if available, to make use of a proper water-bath or oven, kept at a constant temperature by a thermostat. For embedding, small circular metal dishes should be used, slightly wider at the top than at the bottom. These may be made of tin-plate, or, better still, of copper, tinned or nickelled or silvered inside, and

it is convenient, for economy of paraffin, to have three sizes : (1) 2 inches wide by $1\frac{1}{2}$ inches deep ; (2) $1\frac{3}{4}$ inches in diameter and 1 inch deep ; (3) $1\frac{1}{2}$ inches in diameter and $\frac{3}{4}$ inch deep. Each of these should contain paraffin *just* above the melting point.

The object is dropped first into dish (1), then, after a short time (say 15 minutes for a 42-hour chick embryo), it is transferred with a warmed section-lifter to number (2), then, in turn, to number (3). In the case of a 42-hour chick embryo, 5 minutes in each of the pans numbers (2) and (3) should be sufficient. A fourth pan, similar to number (3), should be used for the preparation of the block. This should be full of paraffin so as to delay the process of cooling while the necessary manipulation of the object to get it into position is taking place. Pan number (4) is removed from the water-bath and its under surface cooled for a moment with cold water, so as to cover the bottom of the pan with a thin layer of solid paraffin. The pan is now placed on a couple of slides laid on the table parallel to one another, and a heated electric bulb (preferably with a carbon filament) placed just over the pan, so as to keep the surface of the paraffin melted. The object is now transferred from dish (3), laid on the solid paraffin at the bottom of dish (4), and arranged in position by means of heated needles. If the object be a small animal, destined to be cut into transverse

sections, it should be arranged with its dorsal side below ; if it is destined to be cut into horizontal sections, on the other hand, it should be laid on its side. As soon as the object is properly in position, a little cold water should be squirted under the pan from a pipette to accelerate the solidification of the paraffin from below upwards and so to fix the object in position. The pan may now be floated on a large volume of cold water until thoroughly hard throughout ; it should on no account be submerged before this stage, as this is liable to bring about either the formation of cavities or the crystallization of the paraffin in the interior of the mass. Another advantage of cooling the paraffin from below upwards is that air-bubbles, which are apt to arise during the process of solidification, are able to make their way up into the superficial layer, which will be pared off at a later stage.

When the object is very delicate and liable to shrinkage, it is advisable to interpose between the xylol and the paraffin a bath of a saturated solution of paraffin in cold xylol. Such a solution should be kept in stock. Some of this solution should be warmed on top of the water-bath, and the specimen, after saturation with xylol, transferred to it for a short time before being placed in paraffin.

For very small objects a single bath of paraffin is sufficient. To transfer them to the melted paraffin, they should be taken up from the xylol

tube with a pipette and placed on a section-lifter. The surplus xylol is drained off and the specimen is flicked off into the melted paraffin.

If the object is unstained and of such a nature as not to be conspicuous when embedded in the solid paraffin, it is advisable to stain it temporarily during the process of dehydration by using absolute alcohol saturated with Eosin. This facilitates orientation and the Eosin can easily be removed from the sections later on by treatment with acid alcohol.

Cutting

If the block of paraffin has been cast between L-shaped moulds, slightly smeared with glycerin or vaselin, it is readily detached from them. If, on the other hand, the metal dish has been used, it should be warmed slightly and slowly over a Bunsen flame, so as to loosen the paraffin block. The block is then trimmed down roughly to the desired shape, regard being had to the direction in which the object is to be cut. After attaching the block to the carrier, it should be finally trimmed.

In order to obtain the best results, it will be found worth while to attend to the following points. If transverse sections through a small animal are to be made, the block should be arranged so that the dorsal side is beneath, *i.e.*

next the knife. This is done for convenience in mounting the sections on the slide in the correct position. In the case of many vertebrate embryos it also facilitates the actual cutting, for, if the attempt is made to cut the ventral side first, the hard yolk is apt to furrow and break up the section.

In the final trimming of the block after it is

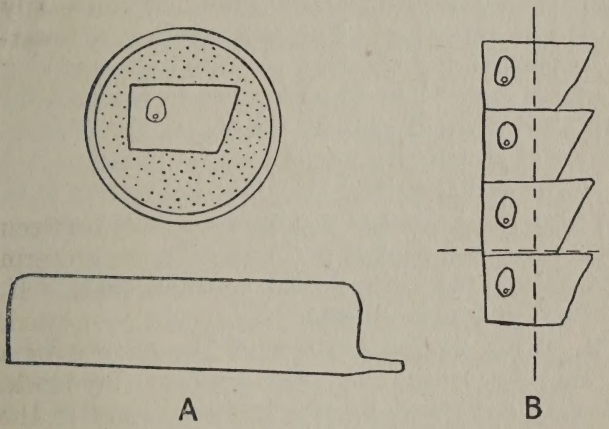

FIG. 6—(A) Diagram to show the arrangement of the block on the carrier with reference to the knife; (B) How to trim the ribbon of sections.

attached to the holder, it will be found advantageous to give it a form like that shown in Fig. 6 (A). It is of advantage, when feasible, to have the paraffin block considerably larger than the object; greater extension in a vertical direction causes the sections to cut better, greater

extension in a horizontal direction cause them to "ribbon" better. The object should be near one corner of the block, to facilitate exact orientation. The upper and lower edges must be exactly parallel, to keep the ribbon straight, and these edges should also be parallel to the edge of the knife. If a coating of soft paraffin is used to make the sections ribbon, it should be confined to the upper and lower surfaces, and should be very thin. The best result is obtained by super-heating the soft paraffin and applying it with a camel-hair brush dipped in the paraffin and then pressed against the lip of the dish to get rid of all but the merest trace of paraffin.

In the actual cutting, the first precaution is to see that the knife has got an exceedingly sharp, even edge, and that it is thoroughly clean. For soft objects the knife should be tilted more than for hard or brittle objects.

If, owing to the dryness of the atmosphere, trouble is caused by the sections becoming strongly electrified by the friction in cutting, a basin of hot water should be placed in the neighbourhood to moisten the air. Sometimes the sections tend to stick to the razor, not through electrification, but simply owing to the temperature being too high.

If, as *occasionally* happens, very thin sections are necessary, say 1μ in thickness, the following procedure should be adopted. Use hard paraffin and thin razor. The block should be trimmed

down so that only a few sections of blank paraffin are cut before entering the object—only sufficient sections to start the ribbon satisfactorily. To do this, it is best to start by cutting a few 5μ sections, then diminishing the thickness in succession to 4μ, 3μ, 2μ, and 1μ. The sections should be cut rapidly, and the ribbon, as it is formed, should be held away from the flat surface of the razor, to which it is apt to adhere, so that successive sections are crushed and compressed by the pressure.

On becoming an expert in section-cutting, there is a considerable temptation to become interested in the production of extraordinarily thin sections, such as 1μ, or even ·5μ. It may therefore be pointed out that such sections are of value only for the solution of certain very special problems. Normally the sections through a particular kind of animal or a particular kind of tissue should be of a thickness comparable with the size of the cells. Thus, in the case of a segmenting egg of a Lung-fish, with its enormous cells, the most instructive sections may be as much as 80μ in thickness! As a general rule, 5μ to 10μ is a suitable thickness for animal tissues, but, in any particular case, experiments should be made with sections of different thicknesses until it is determined which is the most instructive.

In a ribbon of sections prepared in the way indicated there is a large amount of superfluous paraffin which, for the sake of economy, should

be reduced before the sections are mounted. To do this, the ribbon, as it lies on the smooth paper, should be cut along the dotted line of Fig. 6 (B) with a sharp scalpel. The ribbon is cut in similar fashion into short lengths suitable for mounting —each transverse cut being made to pass through the narrower side of the section, so as to avoid injury to the object in case it is not clearly visible in the paraffin section. It should be remembered that the sections are liable to be traversed by minute folds and wrinkles formed parallel to the edge of the razor, and that the flattening out of these causes the diameter of the section to increase in a direction at right angles to the direction of the original wrinkles when treated with warm water. Consequently, each piece of ribbon should be considerably shorter than the space available for a row of sections on the slide, so as to leave a margin for this increase in length during the flattening process.

Albumen

To make up the albumen used for causing the sections to adhere to the slide, the following method should be used. Put into a glass jar 50 cc. of the white of a fresh egg, and beat this thoroughly with a glass rod until it is very frothy. Now add 50 cc. of glycerin and beat the two together. In a watch-glass, dissolve $\frac{1}{2}$ gram of Sodium Salicylate in hot water, and add the solu-

tion to the albumen and glycerin, beating them all together. By this method the mixture is clearer and gives better results than when the three ingredients are all beaten together at once. It will be found also that the albumen keeps better and for a longer time when it is prepared this way. It must now be filtered, and, as the process may take a few months to be completed, the filter should be covered with a bell-jar to keep out the dust.

When at the seaside, albumen can be easily and quickly got by pulling the carapace off a few shore crabs and filtering the liquid found inside the crabs. This is very good albumen, and does splendidly for sticking sections to the slide. To hasten the process of filtration a little saline may be added to the albumen.

CHAPTER VI

The Celloidin Method: Embedding Preliminary Processes

To prepare the specimen for the celloidin method of section-cutting the preliminary processes are the same as for the paraffin method. The specimen is fixed and dehydrated up to absolute alcohol, care being taken that the dehydration is complete. It is of great importance to begin with fresh material, or when for some reason living material is not available, with thoroughly fixed and well-preserved specimens. As a general rule it will be found best to stain in bulk, for two reasons: (*a*) the object is more easily seen and orientated in the celloidin block when it is stained, and (*b*) it is difficult to stain on the slide without also staining the celloidin in the section.

Solutions of Celloidin Required

Four strengths of solution of celloidin should be made up by dissolving the celloidin chips as purchased in a mixture of equal volumes of absolute alcohol and sulphuric ether. The strengths required are 2, 4, 6, and 10 per cent.

solutions, made by dissolving in the proportions of respectively, 2, 4, 6, and 10 grams of celloidin flakes, to 100 cc. of the absolute alcohol and ether mixture. Celloidin, in the form of shreds, is supplied in one-ounce bottles by the Chemische Fabrik (Vorm. E. Schering) at Berlin. The bottles contain one ounce of celloidin shreds, soaked in water. The shreds should be washed in fresh water and then dried, either by spreading them out on blotting paper or by washing them in alcohol, before being dissolved in the absolute alcohol and ether mixture. The simplest way to get a uniform solution is to put the celloidin shreds and the absolute alcohol and ether mixture into a tall jar, then lay the jar on its side, making sure that the shreds are well spread out. Periodically, the bottle should be shaken up and turned over. In this way a solution can be obtained in 24 hours, while, if the bottle is left upright, it would take several days to dissolve the celloidin.

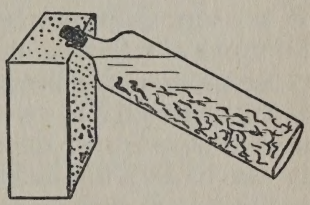

FIG. 7—Method of dissolving shreds of celloidin in the absolute alcohol and ether mixture.

INFILTRATION WITH CELLOIDIN

Having prepared the solutions of celloidin, the carefully dehydrated specimen is transferred from

the absolute alcohol into the mixture of absolute alcohol and ether, and is left therein over-night, or longer, according to its size. It is then transferred to the 2, 4, and 6 per cent. solution of celloidin, being left for *at least two days* in each in turn. Finally, it is put into the 10 per cent. solution and left in it for several days if it is small, up to several weeks if it is large. The specimen is in no way damaged by long immersion in the solutions of celloidin, and the block cuts well *only* if the process of infiltration has not been hurried. If the specimen is in a well-stoppered tube, and has a good quantity of the celloidin solution with it, it can be left untouched during the process of infiltration so that it need not interfere with other work that is being carried out.

Hardening

When the object is ready for hardening, a suitable sized box is made from a postcard or very tough paper, by folding up the sides and ends as is shown in Fig. 8, A, B. Having made the folds *a*, *a'* and *b*, *b'*, the point of intersection of *a* and *b* is caught in such a way that the outer part of *a* lies along the outer part of *b*, and the fold *c* is thus made. Similar folds, *c' c'' c'''* are made at the other corners. The triangular piece thus formed is now folded round the side as in Fig. 8, B. It is well to make the box considerably larger than the specimen, to allow for the orienta-

tion of the block when it has been hardened. The sections always cut best when there is a good margin of spare celloidin round the specimen.

Into the box is poured a fairly thick layer of 10 per cent. celloidin, and the box is placed in a

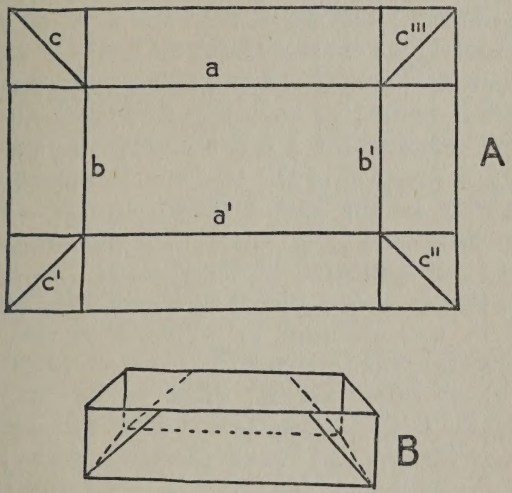

FIG. 8—(A), Diagram to show where to make folds on cardboard; (B), Cardboard box for the preparation of the celloidin block.

well-stoppered bottle with a piece of blotting-paper or cotton-wool saturated with chloroform. The chloroform has the effect of hardening the celloidin, and the box should be left in the chloroform vapour until the celloidin forms a soft jelly. The base of the block so formed must be *solid*, or the fresh celloidin added when the object is put

in will soften the base, and cause the object to sink to the bottom : on the other hand, it must not be *hard*, or a good join will not be made between it and the fresh layer of celloidin. The length of time required varies with the size of the box and the concentration of chloroform vapour in the bottle ; but for a box, the floor of which measures $1\frac{1}{2} \times 1$ inches, about 24 hours is needed.

When the base is hardened sufficiently, its upper surface is moistened with the ether and absolute alcohol mixture for a few seconds, the object is placed in position in the box and it is completely filled with 10 per cent. celloidin and replaced in glass-stoppered jar, with a fresh supply of chloroform saturated blotting-paper. It is best to do this in a cool place, as air-bubbles tend to form in the celloidin as it hardens and heat accentuates this tendency. It will be found that the block takes several days to harden, and, especially with a large block, it is necessary to dry out the jar and renew the chloroform about once in 24 hours, for after a time the ether, evaporating from the celloidin, counteracts the action of the chloroform. A block measuring $1\frac{1}{2} \times 1 \times \frac{3}{4}$ inches will be found to take about 3 days to harden, renewing the chloroform at least once during that time.

Clearing

The hardness required for cutting can only be learned by experience, but a rough test is to try

the hardness of the block with the finger-nail. When an indent is only made with considerable pressure, the hardness is about right for cutting. Unless the block is hardened throughout, the sections will not cut evenly, one section being thick, the next thin.

When the block is hardened sufficiently, it should be cut out of the box with a sharp scalpel and put into a mixture of equal volumes of cedar oil and chloroform, for 3 or 4 hours. It is next transferred to a mixture of cedar oil and chloroform in the proportion of 3 to 1; this may be done by diluting the original half-and-half mixture with an equal volume of cedar oil. The specimen is put into pure cedar oil after 3 or 4 hours immersion in the 3:1 mixture. After a short time the block is given a second bath of pure cedar oil and is left in it for some days until the celloidin is quite transparent. Since the war it has been difficult to obtain absolutely *anhydrous* cedar oil, therefore it is necessary to add 10 per cent. of absolute alcohol to the cedar oil used for clearing, otherwise the block never becomes completely transparent. A large block will need at least 3 or 4 days in the cedar oil to become quite clear and it can be left much longer without any damage.

Trimming the Block

When the block is cleared, it should be trimmed down carefully so as to orientate the specimen as

accurately as possible, at the same time leaving a good margin of spare celloidin round the object to give steadiness when cutting. The specimen is best left as nearly in the middle of the block as possible. The sides of the block should be perfectly smooth, and it is well to have them parallel, the whole block being cubical in form.

CHAPTER VII

The Celloidin Method: Cutting and Mounting

Microtome Carrier

The block, prepared as in the previous chapter, is now ready to be fixed to the carrier. This should be a square or oblong block of hard, white wood, with the base cut into a cylinder to fit the microtome holder. The cylindrical base should be about half the width of the top, to give sufficient rigidity when cutting; it should also be counter-sunk into the top with a square-cut block, only the part below the top being cylindrical. This carrier should be kept in an oven for several days to make sure that it is thoroughly dry. The top of the carrier must be a little rough, to give a hold for the celloidin. When it is ready for use, the top of the carrier should be wet

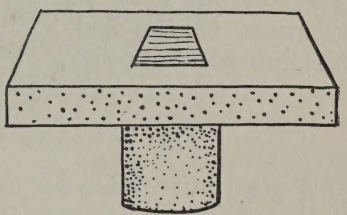

Fig. 9—Wooden carrier for the celloidin block.

with the absolute alcohol and ether mixture, with which the base of the celloidin block should also be moistened. Then a layer of 10 per cent. celloidin is put on the top of the carrier, and the celloidin block is pressed down upon it. Carrier and block are then placed carefully in a glass-stoppered jar, with blotting-paper soaked in chloroform, until the joint is hard. This hardening of the joint will take some hours to be complete throughout; the outer part of the celloidin hardens in a few minutes, but that under the middle of the block, of course, takes longer, and it is best to leave it over-night in the chloroform vapour to ensure that the joint is really secure. The carrier, with its attached block, is then returned to the cedar oil and 10 per cent. of absolute alcohol until such time as it is to be cut.

The Schanze Microtome

A good microtome for celloidin section-cutting is that made by Schanze of Leipzig. This instrument is supplied with a very efficient mechanism for orientating the block accurately. The base of the wooden carrier is placed in the desired position between two metal plates, one of which is movable, and is fixed up tightly with the screw. The wooden carrier should be adjusted so that the side of the celloidin block is parallel with the edge of the knife. The block may now be tilted from side to side by rotating the left hand

adjustive screw, and when the correct angle has been obtained, the position is fixed by pulling its brake-lever firmly towards one. The block is then tilted forwards and backwards—that is, in a direction at right angles to the previous movement—by means of the central, adjusting

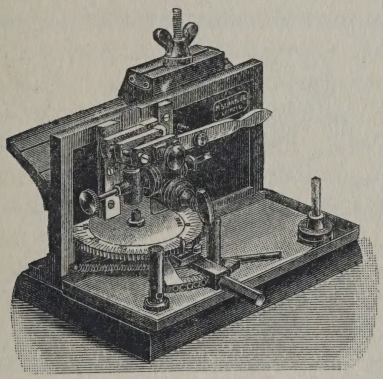

FIG. 10—The Schanze Microtome. The figure illustrates the up-to-date Model B. The description in the text is that of a slightly older model, but in essentials it applies to the present one. The knife-holder of the description is not shown in the figure, but is that supplied by Schanze as knife-holder form C.

screw, and, when the best position is found, the brake-lever alongside this screw is pulled forwards.

Having orientated the block, it is brought into such a position that its upper surface is just touching the lower surface of the razor, by rotating the large disc below the block-carrying

apparatus. The upper surface of the disc is graduated round the circumference, each division of the scale representing 10μ. This disc supports the carrier, which slides in a metal groove, so that by rotating the disc up the carrier is raised, and the thickness of the section to be cut is regulated by the number of scale-divisions through which the disc is moved. The foot of the carrier has

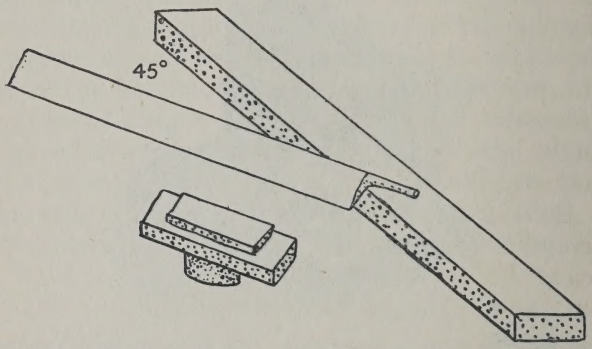

FIG. 11—Diagram to show the position of the knife on the microtome.

attached to it a pointer, to facilitate the regulation of thickness of the sections.

The razor is fixed by two screws into a small socket, the floor of which can be tilted so as to give the razor the slope required. At right angles to the socket which holds the knife this carrier has a U-shaped metal base, which is adjusted at any angle round the upright, with the clamp seen at the top of the figure, on to the top of the

sliding-block by which the razor is carried. The sliding-block is a solid piece of metal with two smooth metal runners along its lower surface and one along the back, which slide on similar metal runners on the top of the microtome.

It will be found that, as a general rule, sections cut best when the knife is fixed in such a position that the back of the knife forms an angle of about 45° with the top of the microtome. The degree of the slope of the razor should, however, be varied until the best angle for the particular block is obtained. The larger the object to be cut is, the greater the slope of the knife should be, *i.e.* the angle indicated in the diagram should be more acute when the block is very large.

Having got the knife in position and the celloidin block accurately orientated, all is now ready for cutting the sections. It is well to have some spare celloidin above the specimen to experiment with as to thickness of section and orientation of the block.

Cutting

The sections are cut by drawing forward the sliding-block, thus bringing the razor across the top of the celloidin block. After each cut, the graduated disc is rotated through the appropriate number of scale-divisions. When a continuous series of sections is required it will usually be found that 25μ is the finest at which they can be

cut, though, with great care, sections as thin as 15μ or even 10μ can be obtained. Usually, however, with large objects, thicker sections are of more use, and it is well to avoid the common fault of section-cutters of trying always to get the thinnest possible sections.

Both the celloidin block and the razor must be kept wet with cedar oil. For this purpose pure cedar oil should be used, not the oil mixed with 10 per cent. absolute alcohol, for the absolute alcohol absorbs moisture from the air so rapidly that the oil begins to form a kind of emulsion on the surface of the block. If the pure oil does not spread evenly over the surface, or if it runs into isolated drops, the block has not been long enough in cedar oil, and it is no use attempting to section it until it has been returned to the absolute alcohol and cedar oil for some time.

The knife must be kept clean and very sharp. In cutting, more especially when very thin sections are wanted, a jerky movement of the knife is advised by Jamieson. The editor has found, however, that a slow, steady movement is best. Each section, as it is cut, must be removed with forceps by drawing it off the razor towards the back. The sections are then arranged in rows in a large flat dish, with enough cedar oil to keep them moist. Between successive cuts, a large drop of cedar oil must be put with a brush on to the top of the celloidin block.

A common difficulty met with is that the sec-

tion rolls up as it is cut. This can sometimes be rectified by cutting thinner sections or by moving the knife more slowly. If neither of these methods stop the curling of the section, the only thing to be done is to brush over the top of the celloidin block towards the razor with the oil-charged brush as the section is being cut. In this way the tendency to roll is overcome, and one soon gets into the way of using the brush with the left hand as one moves the razor forwards with the right. It is important always to have plenty of oil on both the razor and the block.

Sometimes the sections tear badly, and this is usually due to the specimen not having been sufficiently saturated with celloidin before being embedded in the hardened block, or to the block having been too short a time in the cedar oil. It may also be due to the edge of the razor not being good enough, or perhaps some of the screws have worked a little loose, and there is some movement in consequence. It is essential to good results to have both the razor and the block as rigid as possible. If the block is not sufficiently hard, the sections will not cut evenly. They will be, alternately, thick and thin; perhaps a complete section and then a thin shaving. This is due to the elasticity of the block, and it is impossible to cut satisfactory sections; this is more especially the case with large sections. It is therefore necessary to have a very hard block when large sections are to be cut.

Mounting Serial Sections

For mounting serial sections, collodionised slides are needed. These are prepared by dipping clean slides up to about half an inch from the end into collodion, draining off all the surplus liquid, and standing the slides up on end to dry, or laying them horizontally on some kind of rack. It is best, when they are standing upright, to have the clear end on the table and the collodionised end just resting on some support; in this way the film adheres better, for if the collodionised end is down, the film becomes attached to the table, and, in pulling the slide away, the film is apt to be loosened from the slide. The collodion film must be absolutely dry before the sections are mounted on the slide. It takes several hours for the film to dry, so it is best to collodionise the slides the day before they are needed.

The whole series of sections to be mounted should be trimmed to the same size and shape. Each section is then drawn on to a section-lifter and passed through three successive baths of xylol before being laid on the collodionised slide. When the slide begins to dry, a drop of xylol should be added, for the sections will tend to curl up if they are allowed to dry. When the sections are arranged in rows, close together on the slide, any surplus xylol is drained off and, holding the slide slightly on the slope, the absolute alcohol and ether mixture is cautiously dropped from a

pipette on to the middle of each section, following the current down the slide, and only adding *just* sufficient to give every section a slight wetting. This flattens the sections and makes them adhere to the collodionised surface of the slide. Enough absolute alcohol and ether must be used to flatten the sections, but *no more*, or else the sections will not stick, if they are thin; when very thick sections are being mounted (sections of, say, 50μ or more), it will be found that more of the absolute alcohol and ether is needed, as it is necessary to soften the celloidin to make it stick to the collodion film below. Any excess absolute and ether is then drained off, and whenever the slide begins to turn white at the edges, it is plunged into a tube of 90 per cent. alcohol for a minute or so, then for 45 seconds into each of two successive baths of absolute alcohol, and, finally, it is given two baths of xylol, being left in each for about 45 seconds, and then mounted in Canada balsam.

It is very important to use really pure absolute alcohol; a tubeful of absolute alcohol will only dehydrate a few slides and must be renewed frequently. The xylol must also be pure. The dehydrating is one of the most difficult parts of the process, for if the slide is left too long in the absolute alcohol, the sections will come off, and if it is not given long enough the slide becomes white and milky when it is mounted. A very thin solution of Canada balsam is best,

and this is smeared down the middle of the coverslip, as is described, for mounting paraffin sections.

If the specimen has not previously been stained, the slide is taken from the 90 per cent. alcohol into 70 per cent. alcohol, and then the sections are stained with borax carmine in 60 per cent. alcohol (*i.e.* the ordinary weak solution of borax carmine brought up to 60 per cent. strength by adding absolute alcohol and then filtered). Usually, it will be found that the sections stain rapidly, and care must be taken not to over-stain or the celloidin will be coloured also. The slide is then destained and dehydrated in the usual way, cleared in xylol and mounted in Canada balsam. It is better, as has already been pointed out, to stain in bulk, for in the passage through the different liquids in the process of staining on the slide, the sections are very apt to be lost or disarranged.

Mounting Single Sections

When only one section is to be mounted on a slide, the same method as for serial sections may be employed. The section can, however, be mounted on a clean slide, which has not first been coated with collodion. The section, in this case, may be transferred straight from the flat dish on to the slide and covered with a coverslip, using a rather thick solution of Canada balsam.

The thick balsam, in spreading out, tends to flatten out any wrinkles in the section, and this can be assisted by applying gentle pressure to the middle of the coverslip. This method is, of course, only suitable with sections that are already approximately flat; if the section is badly wrinkled, some of the folds will almost certainly remain.

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